Part 1. Veterinary medicine

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INFLUENCE OF BOVINE LEUKEMIA VIRUS ASSOCIATED WITH OTHER VIRAL INFECTIONS ON CATTLE IMMUNITY

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Summary. Comparative hematological and biochemical studies in heifers at the age of 6–8 months with different epizootic background revealed that the persistence of the association of viruses (causative agents of bovine leukemia, infectious bovine rhinotracheitis, bovine parainfluenza-3, and bovine viral diarrhea) negatively affect the status of immunocompetent cells. As the cell number of the macrophage-neutrophil group increases by 25–37%, their functional activity decreases. Significant shifts in the state of protein metabolism, the development of immunosuppression, and intensification of lipid peroxidation processes occur in the bodies of the infected animals, indicating a decrease in the natural resistance of these animals. Significant difference in the intensity of the specific immune response in the vaccinated against pneumoenteritis of the viral etiology animals from BLV-free and BLV-positive farms has not been established.

Keywords: bovine leukemia, infectious bovine rhinotracheitis, bovine parainfluenza-3, bovine viral diarrhea, vaccines, immunity, Ukraine

Bovine leukemia virus (BLV) belongs to the family Retroviridae and closely related to the primate T-lymphotropic viruses types 1–5. It is an oncogenic pathogen that is widespread in cattle globally especially in dairy herds (Polat, Takeshima and Aida, 2017; OIE, 2018). It is known that the persistence of herpes-, paramyxo- and pestiviruses in the herd especially under the conditions of livestock infirmity in relation to bovine leukemia, causes a decrease in the overall resistance of the animal and increases the incidence of infestation (Scott et al., 2006; VanLeeuwen et al., 2001; Konnai, Murata and Ohashi, 2017). It has been proved that the BLV causes an immunosuppression, thereby activating infectious pathology, which in the normal state of the immune system may not manifest itself (Erskine et al., 2012; Frie et al., 2016). It is noted that the increase of the level of infection in young animals with BLV leads to a significant increase of morbidity and mortality due to the activation of respiratory-enteric diseases caused by conditionally pathogenic pathogens (Hopkins and DiGiacomo, 1997; Kobayashi et al., 2015; Buehring, Kramme and Schultz, 1994).

The purpose of this work was to study immunosuppressive changes in the immune system of cattle under herpes-, paramyxo-, pest-, and retroviruses association.

Material and methods. Four groups of animals were formed for the experiments. The number of animals in each group was 10 heads at the age of 6–8 month. The heifers of the first group were selected from the herd, where the circulation of the herpes virus of the first serotype (infectious bovine rhinotracheitis virus, IBRV) was previously confirmed. The clinical manifestation of the disease in the herd was restrained by the preventive vaccination. The second group of heifers was selected from the livestock where the bovine parainfluenza-3 virus (BPIV-3) and IBRV were confirmed by serological study and typical clinical signs of respiratory disorders were observed. Moreover, these farms have had BLV-free status for the last four years. The third group included BLV-positive animals. At the same time, frequent outbreaks of respiratory diseases were observed in the animals of the latter farm, and antibodies against three viruses: BPIV-3, IBRV, and bovine viral diarrhea virus (BVDV), were detected by serological studies of serum samples. The fourth group of animals has been formed from intact individuals.

Serum samples were taken from the animals of each groups for comparative hematological, immunological and biochemical studies.

Number of T- and B-lymphocytes was estimated by hematological studies using the reactions of spontaneous indirect globulin and complement rosette formation, phagocytosis activity, content of large granular lymphocytes, dynamics of cellular changes in the reaction of blast transfer transformations.

Using the serological methods, the presence and titers of antibodies against retro-, herpes-, paramyxo-, and pestiviruses were determined, and the titer of heterohemagglutinins in serum was also determined.

Biochemical studies were performed to determine a total protein, protein fractions, circulating immune complexes of average molecular weight, seromucoids, and rate of lipid peroxidation in the serum.

In order to determine the effect of the cattle leukemia agent circulation on the effectiveness of vaccination against the agents of cattle pneumoenteritis, the level of specific immune response in animals after their inoculation with an inactivated drug containing the agents of IBR, BVD, and BPI-3 has been conducted.

The titer of specific antibodies to the IBRV and BVDV was studied by neutralization reaction, and the titer of specific antibodies to the BPIV-3 by hemagglutination inhibition test. The study of the level of specific antibodies was performed before the inoculation of the drug (start indicators) in 14 days after vaccination of animals, as well as in 30 days, 3 months, and 5 months after administration of the drug. Blood from cows and heifers older than 6 months of age was studied. The indication of IBRV, BVDV, and BPIV-3 was performed by immunofluorescence.

Analysis of the obtained research results was performed using variational methods (Lakin, 1990).

Results. Most animals of the experimental groups had active leukocytosis, which is a significant reaction of the host immune system during the development of acute infectious process. Moreover, in the group of vaccinated animals, which were inoculated with the vaccine against IBRV (the first group), the average leukocyte count exceeds by 8% the same data of the control group (8.75×10^3 and 7.8×10^3 cells/μl respectively). In the third group of calves, where in the chronic course of leukemia, antibodies against BLV to three types of respiratory-intestinal category were detected, the level of leukocytosis in the group average values, on the contrary, was 4% less than in animals control groups (6.73×10^3 and 7.8×10^3 cells/μl respectively).

A similar situation was observed concerning the neutrophil cells. It was found that the quantitative blood counts of the animals from the first two groups were significantly higher than in the control group, whereas in the animals of the third group they decreased.

It should be noted that there is a significant increasing of the number of large granular lymphocytes in the peripheral blood of experimental animals, and a significant decreasing in the number of erythrocytes, as well as the low level of hemoglobin (22% less in animals of the third group and 9% less in animals of the first group) in comparison with the control.

An important indicator of the level of animal resistance is the functional capacity of phagocytes. During the clinical observation of the peripheral blood samples, a significant twofold increasing of the number of monocytes in the first group, moderate in the second group, and a slight decreasing in the third group in comparison with the intact animals were observed.

The most significant phagocytic activity was demonstrated by blood testing in the animals from the first group. In the second and third groups, the phagocytic activity of neutrophils was reduced by 37 and 25% respectively, relative to the control. It was noted that the neutrophils of the animals of the two experimental groups mentioned above had a delayed phagocytosis with a sufficient ratio (25–30%) in peripheral blood samples. The lowest phagocytic and enzymatic activity of neutrophils was observed in animals of the third experimental group against the background of deficiency of these cells and low functional activity.

In addition to the level of neutrophil phagocytosis, the functional activity of immunocompetent cells of the experimental animals was studied using an evaluation of the lymphocyte blast transformation reaction. It is proved that there is a decrease in the functional activity of lymphocytes in all experimental groups, compared to the control animals. It was found that in the animals of the first and second groups the population of T-cells is more active to the immunological loads, while in the animals of the third group the B-lymphocytes are preferred. The animals of the third group had the lowest T-lymphocyte activity and the highest proliferation rate. Generalization of the information obtained as a result of the analysis of the materials of hematological studies, shows that the presence of the association of infectious diseases of different nature, in our case, viruses, provides a significant decrease in the functional activity of immunocompetent cells, resulting in the development of immunodeficiency even in lymphocytosis.

The biochemical study of the serum samples from animals of the first group showed that the level of protein metabolism and the intensity of lipid peroxidation is almost indistinguishable from similar indicators of calves in the control group (Table 1).

This applies primarily to indicators of total protein, protein fractions, average molecular circulating immune complexes and suppressor proteins (seromucoids). Within the first experimental group, it was found that the level of the lipid peroxidation — conjugated dienes increased by an average of 69.3% compared to the limit of their level in the control animals which might be explained by vaccination as a compensatory response.

Analysis of the biochemical parameters in serum of animals from the second group, where two pathogens (IBRV and BPIV-3) circulated, with moderate manifestation of the infectious process, shows that there is no significant deviation of their level from the control one.

The study of serum indicators in animals of the third group showed a decreasing of the total proteins level and albumins by an average of 14.4 and 23.8% ($p \le 0.05$) compared to the indicators of the control group. It might be caused by inhibition of protein-synthetic function of the liver.

Table 1 — Biochemical parameters in the serum samples from calves of experimental and control groups (M ± m, n = 10)

Variables		Groups			
		I	II	III	IV
Total proteins, g/l		71.8 ± 2.1	75.8 ± 1.9	67.6 ± 2.8*	79.0 ± 6.0
Albumins, g/l		32.2 ± 1.1	32.9 ± 1.2	25.5 ± 1.5*	33.3 ± 3.4
Globulins, g/l		46.8 ± 0.05	42.6 ± 2.0	42.1 ± 2.4	38.8 ± 2.6
Albumins/globulins ratio		0.84 ± 0.05	0.71 ± 0.06	0.67 ± 0.12*	0.90 ± 0.03
Circulating immune complexes, mg/ml		0.110 ± 0.005	0.10 ± 0.04	0.12 ± 0.01	0.16 ± 0.04
Seromucoids, mg/ml		0.20 ± 0.005	0.19 ± 0.01	0.25 ± 0.01*	0.15 ± 0.03
Lipid peroxidation process activity	Conjugated dienes, μmol/l	50.8 ± 0.4*	39.5 ± 0.92	56.1 ± 1.05*	30.0 ± 4.0
	Malondialdehyde, ΔD	9.2 ± 0.1	8.1 ± 0.24	10.0 ± 0.27*	7.5 ± 0.5

Note: * — $p \leq 0.05$ compared with healthy cows.

On the other hand, there was a significant increasing of the seromucoids concentration by 66.7% in the blood of these animals compared to the control level, which is a sign of pronounced development of immunosuppression in the experimental calves. At the same time, excessive accumulation of membrane-altering toxic products such as conjugated dienes and malondialdehyde were registered in the animals of this group, which in averages is 187.0 and 133.3% ($p \leq 0.05$) respectively in compare to the control values. This indicates the intensification of the lipid peroxidation processes and is a sign of impaired functional and structural state of the host cells, including immune system. The established nature of changes in biochemical parameters in the blood of the test calves is consistent with the serological monitoring of the disease of this stock from such viruses as IBRV, BPIV-3, BVDV, and BLV and it illustrated the pathogenetic shifts that accompany the development mentioned above.

It was determined that in the groups of animals in which the above association of viruses circulates, within 2 weeks after the second inoculation of the inactivated virus vaccine against IBRV, BVDV, BPIV-3 both in the BLV-free farms and in those where cattle leukemia is registered, an increase in the level of specific antibodies to identified pathogens was noted.

It should be noted that it was exactly the period of the maximum raise of the level of specific antibodies, which was for IBRV 4.2 ± 0.4 $\log_2$ for the BLV-free farm (Fig. 1) and 3.8 ± 0.6 $\log_2$ for the BLV-positive farm (Fig. 2); for BVDV virus — 3.0 ± 1.0 and 3.2 ± 0.8 $\log_2$ respectively, for the pathogen of BPIV-3 — 5.2 ± 1.2 and 5.0 ± 1.0 $\log_2$ respectively.

Analysis of clinical and epizootic examination of animals from both farms revealed that no new cases of animal disease were detected in both farms. In general, observing the vaccinated animals for five months, it was noted that the use of inactivated drugs for specific prevention of pneumoenteritis, both in farms where the circulation of BLV was detected and in free from the causative agent farms, had a positive effect on epizootic situation concerning respiratory and intestinal diseases. Regarding the dynamics of the level of specific antibodies in vaccinated animals, it was noted that in both farms the use of the vaccine provided a stable high level of specific immune response to the administration of the drug. Thus, after the maximum rise in the level of specific antibodies two weeks after the vaccination of animals from both farms, we recorded a consistently high level of them, ranging from one month to 5 months (observation period) after vaccination.

It should be noted that in free from BLV farm, the level of specific antibodies to IBRV was 4.6–3.2 $\log_2$, and in the retrovirus-positive farm — 4.4–3.6 $\log_2$; to BVDV — 3.0–3.4 $\log_2$ (in both farms); and to the causative agent of BPIV-3 — 5.2–6.0 $\log_2$ (in BLV-free farm) and 5.0–5.4 $\log_2$ (BLV-positive farm) (Fig. 2).

Due to the fact that serological diagnostics is an indirect method of indicating pathogens, we determined the presence of infectious pneumoenteritis pathogens circulation in biological material from animals kept in farms with different epizootic BLV-status.

It was found that, three months after vaccination of the animals in BLV-free farm, the antigens of the IBRV, BVDV, and BPIV-3 were detected in individual animals. Conducting similar studies in animals from BLV-positive farm we also identified the circulation of IBRV and BPIV-3 in individual animals.

Analyzing the epizootic situation in both farms, it was found that manifestation of the disease of animals with respiratory syndrome was not observed, the mortality of animals due to infectious pathology was not detected. Examining samples of biological material selected from vaccinated animals 5 months after vaccination, it was found that both in the animals kept in the BLV-free farm, and in the BLV-positive herd the IBRV, BVDV, and BPIV-3 were not detected.

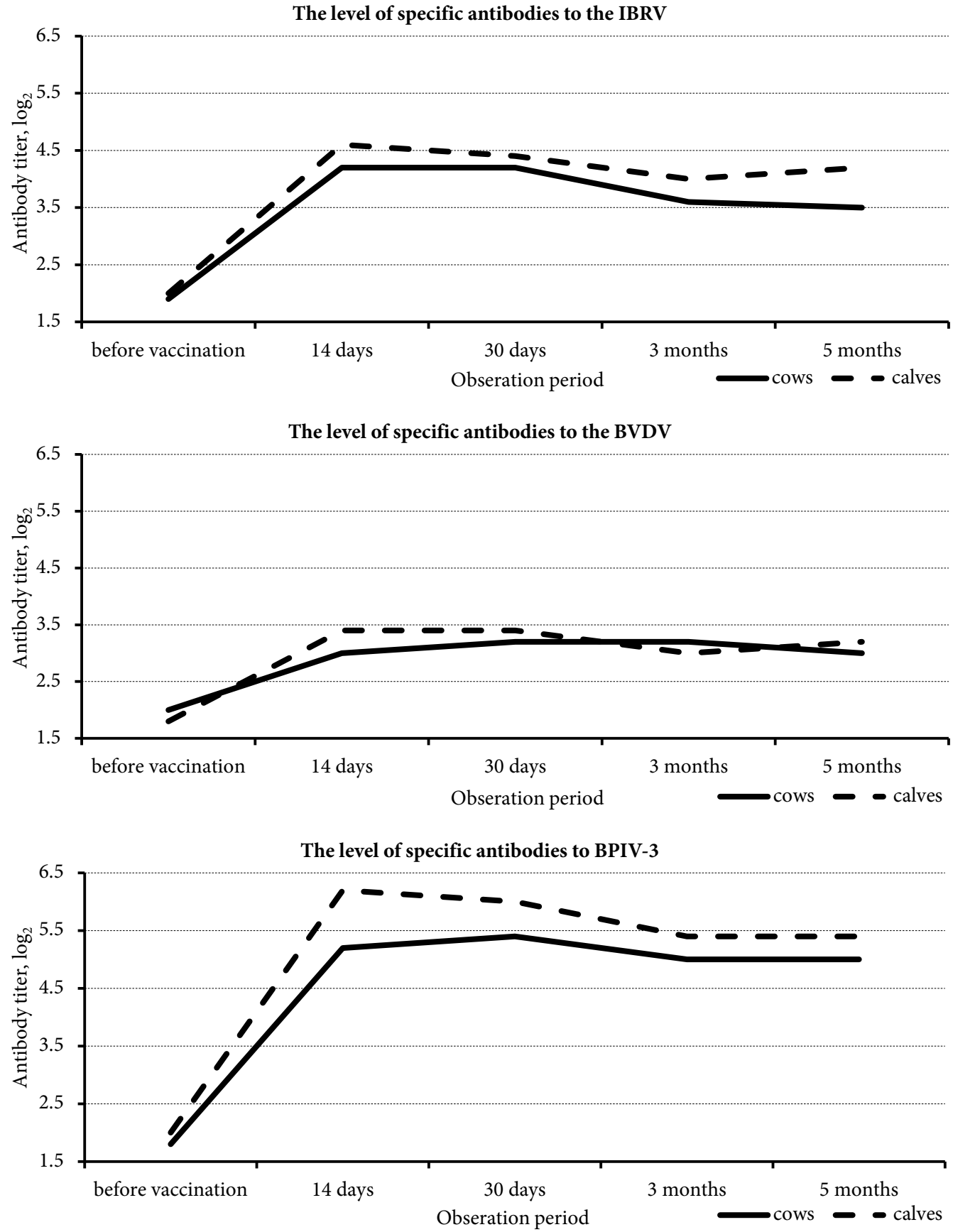

Figure 1. Dynamics of the intensity of specific immune response to the agents of IBRV, BVDV, and BPIV-3 long time after vaccination in the BLV-free farm.

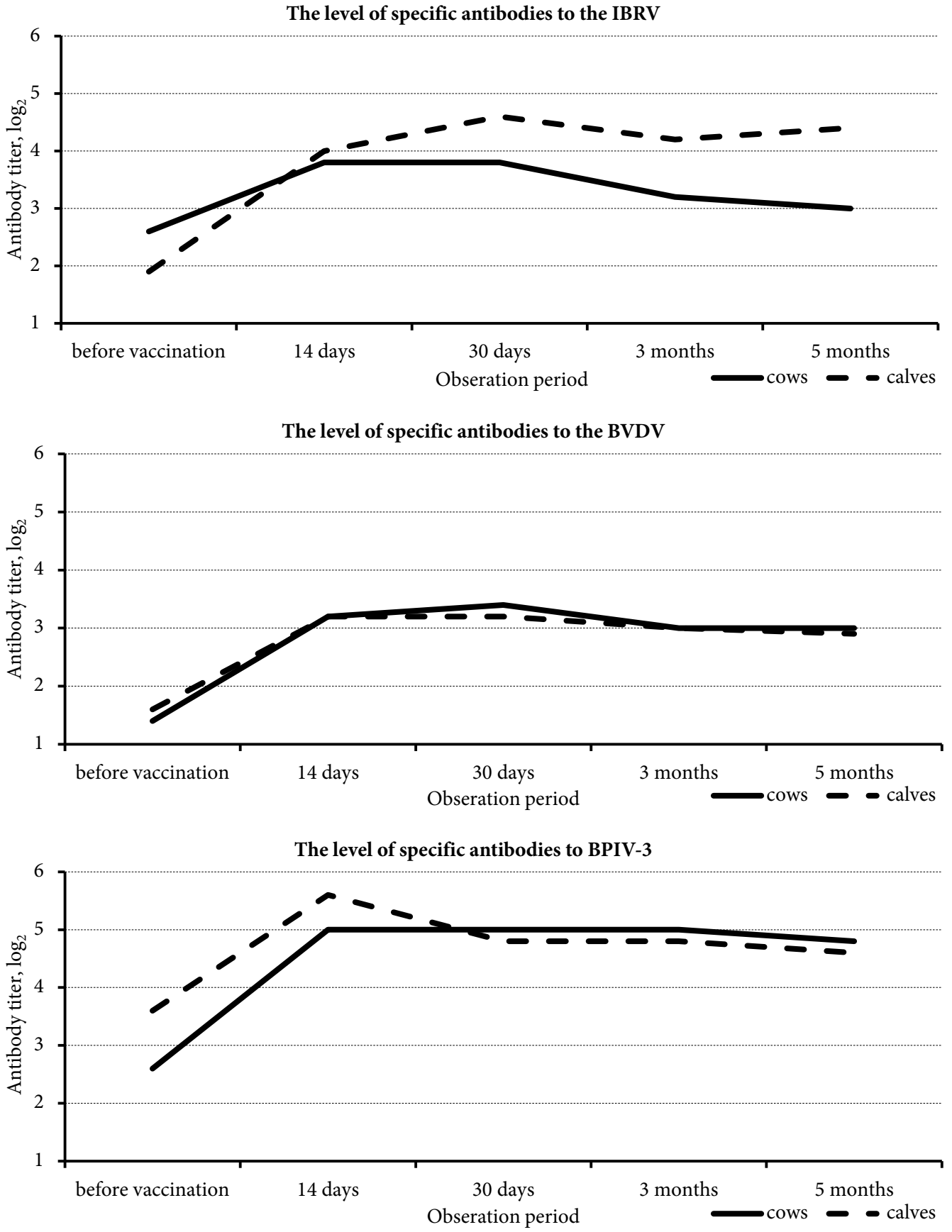

Figure 2. Dynamics of the intensity of specific immune response to the IBRV, BVDV, and BPIV-3 long time after vaccination in the BLV-positive farm.

Conclusions. 1. It is established that in experimental animals on the background of increasing cell number of macrophage-neutrophil group there is a significant (25–37%) decreasing in their functional and enzymatic activity. The decreasing of the functional activity of immunocompetent cells is significant during the BLV, IBRV, BVDV, and BPIV-3 persistence in animals.

2. In infected animals (BLV, IBRV, BVDV, and BPIV-3) there are significant changes in the state of protein metabolism, development of immunosuppression and intensification of the processes of lipid peroxidation, which indicates a decreasing of the natural resistance in these animals.

3. According to the results of the comparative determination of the effectiveness of specific prevention of cattle pneumoenteritis using inactivated vaccine, which was introduced in BLV-negative, and BLV-positive herds, there was found that epizootic status of farms regarding retrovirus did not have a significant effect on the intensity of specific immunity and the effectiveness of ensuring sustainable well-being regarding IBRV, BVDV, and BPIV-3.

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